Putting Peptides to Work

When people think about amino acids, they may worry about getting their daily nutritional requirement to build and maintain muscle. For the more biologically sophisticated thinker, amino acids are the building blocks of peptides and proteins, which are the primary effectors of our genetic code—the enzymes and transporters and regulators of cellular function in health and disease. When Joel Schneider, Ph.D., Chief of CCR's Chemical Biology Laboratory, thinks about amino acids, he sees them as the building blocks of new materials. From his research into the fundamental mechanisms of peptide folding, he explores novel ways to address challenging medical needs. Research in his laboratory has applications ranging from tissue repair to drug delivery.

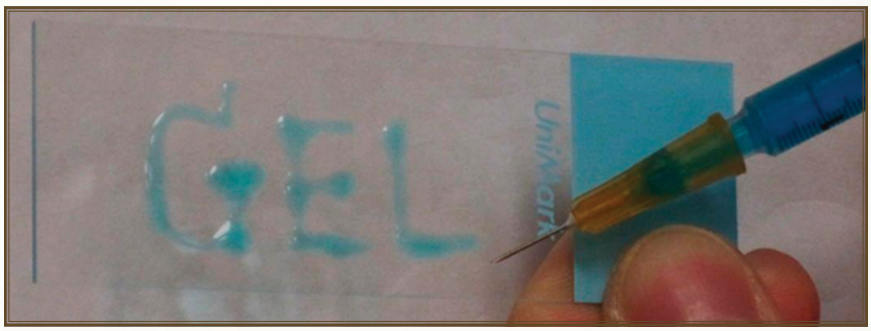
Lending a Hand

These days, cutting off a hand is not irreversible. Gerald Brandacher, M.D., Scientific Director of the Composite Tissue Allotransplantation Program at the Johns Hopkins Medical Institute, for example, specializes in reconstructive transplantation, such as whole-hand transplants. Donor hands are flown in and painstakingly attached, micrometer-wide blood vessel by blood vessel, to the recipient's forearm. "But there's a problem," explained Schneider, who is actively collaborating with Brandacher to improve this procedure. "The blood vessels are collapsed in the donor hand. It's like taking a hollow spaghetti noodle that has collapsed and trying to stitch it to another."

Dan Smith, a graduate student in Schneider's laboratory, earning his Ph.D. from the University of Delaware, is developing a gel that can be injected into the donor hand's vessels to effectively plump them up. The concept sounds simple, in principle, but the material has to be able to transition from a semi-solid gel to a viscous liquid that can be delivered smoothly to the lumen of the vessel through a syringe. However, once delivered, the material must transition back into the original gel that will fill out and support collapsed blood vessels once it is in place. (This sought-after quality—shear thinness—is the property that turns ketchup from a thick syrup into a free-flowing fluid with a squeeze of the bottle). Then, after the surgeon sutures the vessels together, the gel must undergo yet another phase transition forming a liquid so that the introduction of circulating blood at the end of the procedure can carry it away.

This anastomosis gel that Smith and Schneider are developing is made from peptides. "We use and design peptides that form fibrous molecular networks, in other words, gels," explained Schneider, "We have designed gels that are self-assembling, shear-thinning, and self-healing."

Like all good designers, Schneider and his team operate from a set of core principles that motivate a prototype and then they iteratively refine the material until it exactly suits their purpose. Schneider's academic career traces its roots to the study of protein folding and the prediction of that folding from amino acid sequences. "We work from our knowledge of protein structure—rules that have been established by ourselves and others—to design materials *de novo*. Often, we initially design something that's not quite what we are shooting for, but we learn from it and improve on it. It's an iterative process."



(Image: J. Schneider, CCR)

A syringe deliverable shear-thinning peptide gel encapsulating a blue dye for visualization

(Photo: R. Baer)

Cem Sonmez, Joel Schneider, Ph.D., Michael Giano, and Katelyn Nagy

Healing Deep Wounds

Proteins are ancient adhesives. According to Wikipedia, the oldest known bow for hunting was constructed some 10,000 years ago using glue boiled down from animal hoof protein. When a wound heals naturally, cells lay down a matrix of proteins, along with carbohydrates to form an extracellular matrix (ECM), a sort of adhesive that holds tissue together. Schneider's group has been developing novel peptide gels that mimic native ECM in efforts to enhance the wound-healing process. These gels are designed to provide the scaffolding for cells until they can remodel and rebuild the wound site.

In separate work that also involves the ECM, Postdoctoral Fellow Yuji Yamada, Ph.D., is developing a new bioadhesive that is made from two primary components: a carbohydrate and a therapeutic protein. When they are mixed together from a dual-barrel syringe (much the same technology as used for epoxy from a hardware store), they form an adhesive that chemically bonds with the ECM. The carbohydrate portion of the gel is responsible for the chemical bonding, while the protein acts as the crosslinker that defines the gel. As the material degrades, it is designed to slowly release the therapeutic protein locally to the tissue.

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Mike Giano, a graduate student in the lab, has recently replaced the protein component of the gel with a polyamine polymer and the result is a bioadhesive polymer that is extremely antibacterial. At physiological pH, polyamines are positively charged—polycationic—which makes them toxic to microbes, including gram-positive and gram-negative bacteria, both of which Giano has tested *in vitro*. Mammalian cells, on the other hand, are unaffected by these polycationic surfaces. "The idea is to use these bioadhesive gels as wound fillers, for example, after tumor resection. The gel would not only help maintain structural integrity of the tissue as it heals, it would limit opportunistic infections," said Schneider.

Delivering and Releasing Drugs

Basic cancer biologists focus on identifying drivers of disease. But, the development of molecules that can effectively target those drivers is as great a scientific challenge [See "A Rich Legacy and a Bright Future"]. Over 30 percent of small-molecule drugs, over 90 percent of approved anticancer drugs, and nearly all protein therapeutics cannot be delivered orally. Instead, they are delivered parenterally, meaning via injection into the blood stream, into muscle, or under the skin. Schneider's laboratory is developing materials to facilitate those delivery modes in order to improve patient compliance by lowering dosing frequency, improving efficacy, and ameliorating toxicity.

One of the key attributes of many of Schneider's new materials is reversibility. Whether for blood vessel anastomosis or wound healing, the gel should not persist indefinitely. Reversibility also confers the possibility of controlled, slow-release drug delivery. With several classes of materials, it is possible to encapsulate even living cells and use the gel as a delivery vehicle to localize the therapy to the tissue before releasing it. "At NCI, of course, we are interested in delivery to tumors," said Schneider. "Imagine localizing a highly toxic small molecular therapeutic directly to a tumor while sparing healthy tissue."

Schneider's group is particularly interested in developing materials that can release interleukins at very slow, known rates. Interleukins are key modulators of the immune system, whose precise location and concentration are critical to their action. In collaboration

with Scott Durum, Ph.D., Deputy Chief of CCR's Laboratory of Molecular Immunoregulation, and Scott Walsh, Ph.D., University of Maryland Assistant Professor, Schneider's group is working on a material that can release minute amounts of IL-7 over time to stimulate T cell-mediated tumor clearance. Such a material could be introduced after a tumor resection to enhance immune surveillance and discourage recurrence.

Currently the project rests on Schneider's team developing a material that can release IL-7 with a consistent profile *in vitro*. Walsh, a protein biochemist, has previously developed methods to express IL-7 in large quantities, a prerequisite to developing the technology. And Durum, an immunologist with a longstanding interest in the mechanisms underlying IL-7's effects on T cells, has developed the animal models to test the material once it is refined.

"Honestly speaking, a lot of collaborations come about because people are friends," explained Schneider. "Scott Walsh and I were lab mates at the University of Pennsylvania. We got together for dinner one evening and just started talking about our work and hit upon the controlled release idea. Scott's ongoing collaboration with Durum, here at NCI, brought us full circle."

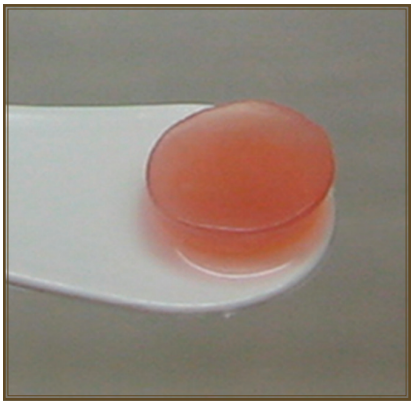
Developing the Basics

Prior to joining CCR, Schneider developed an interest in tissue engineering, with a focus on rebuilding cartilage. At a basic level, he and his team continue to study how different cell types interact with their peptide-based materials. They are exploring whether it is possible to design a material that is conducive to the growth of a particular cell type.

They have now developed a suite of materials that are more conducive to maintaining chondrocytes, the key cells that produce and maintain cartilage. By encapsulating cells within gels and then using the gels' shear thin capacity to conform the cells to molds, the team can study how the cells respond to the encapsulation, and how the nanostructure of the gel network affects the cells' ability to lay down new cartilage.

(Photo: R. Baer)

Dan Smith and Joel Schneider, Ph.D.



(Image: J. Schneider, CCR)

Chondrocytes embedded within a peptide gel produce cartilage

At an even more basic level, Schneider's group is furthering their abilities to predictively design new materials by studying the fibril network structures they produce, using a myriad of microscopy and spectroscopy techniques. Their laboratory is based at the NCI campus in Frederick, Md., which is NCI's hub for chemistry and physical sciences. In addition to chemical biology, the Frederick campus hosts high-throughput screening, structural biophysics, NMR spectroscopy, x-ray crystallography, and the world's largest, most diverse public natural product repository.

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Unique characteristics notwithstanding, the two campuses maintain strong ties. "To be surrounded by biologists and clinicians is truly a gift that enables and inspires your own research program as a chemist. When you are a faculty member of a traditional arts-and-sciences department at a university, you don't have the opportunity to rub elbows with physicians who have problems that need solving," said Schneider.

To learn more about Dr. Schneider's research, please visit his CCR Website at http://ccr.cancer.gov/staff/staff.asp?name=jschneider.

A Rich Legacy and a Bright Future

"NCI has a very old and rich history of chemistry-based research," explained Joel Schneider. "As far back as 1968, chemical research was an integral part of the Drug Development Branch. Later, chemistry was formalized with the creation of the Laboratory of Medicinal Chemistry, first headed by John Driscoll and later by a wonderful chemist named Victor Marquez."

When Marquez retired, the NCI leadership tapped Schneider, then a Professor at the University of Delaware, to extend CCR's reach beyond classical medical chemistry and forge a team of investigators focused on chemical biology. "Medicinal chemistry is necessary to bring a drug to the clinic. NCI was looking to maintain that ability but also to take advantage of the new tools and resources enabled by chemical biology to inform and hasten the discovery and development process," said Schneider.

After rising through the career ranks in a university setting, Schneider was ready for a new administrative and scientific challenge. He quickly hired three new investigators—Jay Schneekloth, Ph.D., Martin Schnermann, Ph.D., and Jordan Meier, Ph.D.—to run their own research programs in chemical genetics, organic synthesis, and chemical genomics, respectively. "The main criteria was to hire outstandingly smart people," said Schneider. "At the end of the day, research evolves, so I didn't want to hire folks based on their ability to fill a specific research niche that is hot now, but rather hire people that can identify opportunities, adapt to change, and make significant impact."

Schneider also established a synthetic core facility within the Chemical Biology Laboratory with full-time chemists who are charged with helping CCR investigators solve chemistry-based problems. "Our laboratory is surrounded by a sea of non-chemist investigators who need non-commercially available molecules to further their research. Before the core was established, they would ask us for help, but we didn't have the resources to divert away from our own research." Now, through the synthetic core facility, any CCR investigator can access the chemistry expertise he or she needs.

Susan Bates, M.D., a Senior Investigator in the Developmental Therapeutics Branch, recently took advantage of the facility to synthesize compounds for preclinical testing. Her team needed dual pathway inhibitors and discovered that they were not readily available. "We found two in the literature," recounted Bates. "In the case of one compound, the company that developed it did not have it on hand, but told us how to synthesize it. The other compound was not commercially available, but it was publicly reported so the core facility chemists were able to develop the synthesis. Dr. Schneider's group produced both compounds rapidly and of good quality. We already have some nice data with them. The core facility is a great resource for biologists and clinicians with chemistry-based needs."

(Photo: B. Branson)

Jay Schneekloth, Ph.D.

(Photo: B. Branson)

Martin Schnermann, Ph.D.

(Photo: B. Branson)

Jordan Meier, Ph.D.